considered
/Kevin K. Hill/
September 21, 2010

Patent Application
Attorney's Docket No.: 1DE10639P00080US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sudhir Agrawal, Lakshmi Bhagat, Dong Yu and Ekambar R. Kandimalla		
Application No.:	10/757,345	Group:	1633
Filed:	January 14, 2004	Examiner:	Hill, Kevin Kai
Confirmation No.:	3490		
For:	MODULATION OF IMMUNOSTIMULATORY PROPERTIES OF OLIGONUCLEOTIDE-BASED COMPOUNDS BY UTILIZING MODIFIED IMMUNOSTIMULATORY DINUCLEOTIDES		

****

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 or is being facsimile transmitted to the United States Patent and Trademark Office on the date indicated below.

Date: September 17 2010

Signature: Joseph C. Zuckerb

Printed Name: Joseph C Zuckerb

****

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Dear Sirs:

I, Ekambar Kandimalla hereby declare as follows.

1. I am employed by Idera Pharmaceuticals, Inc. in the position of Vice President of Discovery. A copy of my *Curriculum vitae* is attached as Exhibit 1.

2. I understand that the Office Action mailed from the US PTO on April 16, 2010, interprets the terms "P-base" and "dP" to describe a genus and, absent to evidence to the contrary, the instantly claimed pyrrolo-[2,3-d]-pyrimidine nucleoside analog species, 2-oxo-7-deaza-8-methyl purine is an art recognized species thereof.

3. As shown in Exhibits 2-4, P-base or, alternatively, dP are common names used to identify a particular nucleotide having the structure:

P Base

Therefore, the terms P-base or dP do not represent a genus of nucleotides.

5. Furthermore, as shown in Exhibits 5-6, Pyrrolo-dC is the common name used to identify a distinct nucleotide having the structure:

Pyrrolo Base

6. Although Figure 4 of Woo et al. (Nucleic Acids Res. 24(13):2470-2475, 1996) depicts a nucleotide having the structure of pyrrolo-dC and labels this nucleotide as dP, as demonstrated by exhibits submitted herewith, the labeling of the pyrrolo-dC nucleotide as "dP" by Woo et al. was in error.

7. I hereby further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: 

Ekambar R. Kandimalla, Ph.D.

Dated: Sep. 17, 2010.

EKAMBAR R. KANDIMALLA
Idera Pharmaceuticals, Inc.
167 Sidney Street, Cambridge, MA 02139.
Tel 617-679-5536; email ekandimalla@iderapharma.com

Employment

01/2008 – Present	Vice President of Discovery, Idera Pharmaceuticals.
07/2003 – 12/2007	Senior Director of Research, Idera Pharmaceuticals (formerly Hybridon, Inc.). TLR targeted immunotherapeutics.
08/1999 – 06/2003	Director of Antisense and Functional Genomics, Hybridon, Inc. Application of antisense technology for functional genomics - Antisense oligonucleotide design, synthesis and target validation, fluorescence based PCR probes and primers, CpG-oligodeoxynucleotide-ased immunotherapeutics, preclinical studies of antisense oligos.
07/1993 - 07/1999	Sr. Research Scientist, Hybridon, Inc. Design, synthesis, biophysical and biochemical studies of modified antisense and triplex-forming oligos; Studies of the interaction of oligos with biological macromolecules; Solid phase attachment of oligos for diagnostic and analytical uses.
06/1992 - 06/1993	Research Scientist, Hybridon, Inc. Design, synthesis, biophysical and biochemical studies of modified antisense oligonucleotides
09/1987 - 06/1992	Research Associate, Department of Chemistry, University of Alberta. Molecular recognition of nucleic acids; Design, and synthesis of sequence specific minor groove binding peptide antibiotics as anticancer and gene expression control agents; Structural aspects of modified RNA and DNA oligonucleotides; Biophysical, biochemical and molecular biological studies on DNA-binding agents and proteins.
01/1985 - 09/1987	Research Associate, Molecular Biophysics Unit, Indian Institute of Science. Design, synthesis and nucleic acid binding studies of new analogs of DNA binding peptide antibiotics netropsin and distamycin.
02/1981 - 12/1984	Jr. and Sr. Research Fellow, School of Chemistry, Andhra University. Graduate student.

Education

Ph.D.	Chemistry, Andhra University, India	1984
M.Sc.	Biochemistry, Andhra University, India	1980
B.Sc.	Chemistry (Major) & Botony and Zoology, Andhra University, India	1978

Selected Bibliography

105. D.Wang, FG.Zhu, L.Bhagat, Y.Li, D.Yu, JX.Tang, **ER.Kandimalla** & S.Agrawal. Study of Toll-like receptor 9 antagonists in lupus prone MRL-lpr mouse model. Proc Natl Acad Sci USA (accepted for publication).
104. D.Yu, MR.Putta, L.Bhagat, Y.Li, FG.Zhu, D.Wang, JX.Tang, **ER.Kandimalla** & S.Agrawal. Agonists of Toll-like receptor 9 containing synthetic dinucleotide motifs. *J. Med. Chem.* 50, 6411-6418, 2007.

103. S.Agrawal & **E.R.Kandimalla**. Synthetic agonists of Toll-like Receptors 7, 8, and 9. *Biochem. Soc. Trans.* **35**, 1461-1467, 2007.

102. **T.Lan, ER.Kandimalla**, D.Yu, L.Bhagat, Y.Li, D.Wang, FG.Zhu, JX.Tang, MR.Putta, YP.Cong, AF.Trombino, T.Sullivan & S.Agrawal. Stabilized immune modulatory RNA (SIMRA) compounds as agonists of Toll-Like Receptors 7 and 8. *Proc. Natl. Acad. Sci. USA*. **104**, 13750-13755, 2007.

101. V.Damiano, R.Caputo, S.Garofalo, R.Bianco, R.Rosa, L.Racioppi, G.Merola, S.DePlacido, G.Fontanini, **ER.Kandimalla**, S.Agrawal, F.Ciardiello & G.Tortora. Novel TLR9 agonist synergizes by different mechanisms with bevacizumab in sensitive and cetuximab-resistant colon cancer xenografts. *Proc. Natl. Acad. Sci. USA*. **104**, 12468-12473, 2007.

100. FG.Zhu, **ER.Kandimalla**, D.Yu & S.Agrawal. Oral administration of a synthetic agonist of TLR9 potently modulates peanut-induced allergy in mice. *J. Allergy Clin. Immunol*. **120**, 631-637, 2007.

99. CS.Ebert, Jr., AS.Rose, DA.Blanks, RP.Eapen, **ER.Kandimalla**, S.Agrawal & J.Prazma. Immunomodulatory oligonucleotides in prevention of nasal allergen-induced Eustachian tube dysfunction in rats. *Otolaryngol Head Neck Surg*. **137**, 250-255, 2007.

98. DA.Blanks, CS.Ebert, Jr., RP.Eapen, **ER.Kandimalla**, S.Agrawal & J.Prazma. The Immunomodulatory Oligonucleotides in the Prevention and Treatment of OVA-induced Eustachian Tube Dysfunction in rats. *Otolaryngol Head Neck Surg*. **137**, 321-326, 2007.

97. EC.Lacasse, GG.Cherton-Horvat, KE.Hewitt, LJ.Jerome, SJ.Morris, **ER.Kandimalla**, D.Yu, H.Wang, W.Wang, R.Zhang, S.Agrawal, JW.Gillard & JP.Durkin. Preclinical characterization of AEG35156/GEM640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. *Clin. Cancer Res*. **12**, 5231-5241, 2006.

96. CS.Ebert Jr, AS.Rose, MR.Patel, SM.Hardy, **ER.Kandimalla**, S.Agrawal, J.Prazma, & HC.Pillsbury 3rd. The role of immunomodulatory oligonucleotides in prevention of OVA-induced Eustachian tube dysfunction. *Int. J. Pediatr. Otorhinolaryngol*. **70**, 2019-2026, 2006.

95. H.Wang, ER.Rayburn, W.Wang, **ER.Kandimalla**, S.Agrawal & R.Zhang. Immunomodulatory oligonucleotides as novel therapy for breast cancer: Pharmacokinetics, in vitro and in vivo anticancer activity, and potentiation of antibody therapy. *Mol. Cancer Ther*. **5**, 2106-2114, 2006.

94. H.Wang, ER.Rayburn, w.Wang, **ER.Kandimalla**, S.Agrawal & R.Zhang. Chemotherapy and chemosensitization of non-small cell lung cancer with a novel immunomodulatory oligonucleotide targeting Toll-like receptor 9. *Mol. Cancer Ther*. **5**, 1585-1592, 2006.

93. MR.Putta, FG.Zhu, Y.Li, L.Bhagat, YP.Cong, **ER.Kandimalla** & S.Agrawal. Novel oligodeoxynucotide agonists of TLR9 containing N3-Me-dC or N1-Me-dG modifications. *Nucleic Acids Res*. **34**, 3231-3238, 2006.

92. D.Trabattoni, A.Clivio, DH.Bray, L.Bhagat, S.Beltrami, G.Maffeis, E.Cesana, P.Lowery, F.Lissoni, **ER.Kandimalla**, T.Sullivan, S.Agrawal, R.Bartholomew & M.Clerici. Immunization with gp120-depleted whole killed HIV immunogen and a second-generation CpG DNA elicis strong HIV-specific responses in mice. *Vaccine*, **24**, 1470-1477, 2006.

91. EC.Lacasse, **ER.Kandimalla**, P.Winocour, T.Sullivan, S.Agrawal, JW.Gillard & J.Durkin. Application of XIAP antisense to cancer and other proliferative disorders: Development of AEG35156/GEM®640. *Ann. NY Acad. Sci*. **1058**, 215-234, 2005.

90. **ER.Kandimalla**, L.Bhagat, Y.Li, D.Yu, D.Wang, YP.Cong, SS.Song, JX.Tang, T.Sullivan & S.Agrawal. Immunomodulatory oligonucleotides containing cytosine-phosphate-2'-deoxy-7-deazaguanosine motif as potent TLR9 agonists. *Proc. Natl. Acad. Sci. USA*. **102**, 6925-6930, 2005.

89. Y.Li, **ER.Kandimalla**, D.Yu, JX.Tang, & S.Agrawal. Immunomodulatory oligonucleotides containing synthetic CpR and R'pG motifs augment long-term immune responses to HBsAg in mice. *Int. Immunopharmacol*. **5**, 981-991, 2005.

88. D.Wang, **ER.Kandimalla**, D.Yu, JX.Tang & S.Agrawal. Oral administration of second-generation immunomodulatory oligonucleotides induce mucosal Th1 immune responses and adjuvant activity. *Vaccine* **23**, 2614-2622, 2005.

87. S.Agrawal & **ER.Kandimalla**. Roll of Toll-like receptors in antisense and siRNA, Nat. Biotechnol. **22**, 1533-1537, 2004. Corrigenda: *Nat.Biotechnol*., **23**, 117, 2005.

86. DC.McManus, CA.Lefebvre, GC.Horvat, M.St.-Jean, **ER.Kandimalla**, S.Agrawal, S.Morris, JP.Durkin & EC.LaCasse. Loss of XIAP protein expression by RNAi and antisense approaches sensitizes cancer cells to functionally diverse chemotherapeutics. *Oncogene* **23**, 8105-8117, 2004.

85. JL.Bjersing, A Tarkowski, **ER Kandimalla**, H.Kalsson, S Agrawal & LV.Collins. Impact of site-specific nucleobase deletions on the arthrogenicity of DNA. Inflammation. **28**, 159-168, 2004.

84. FG.Zhu, **ER.Kandimalla**, D.Yu, JX.Tang. Modulation of ovalbumin induced Th2 responses by second generation immunomodulatory oligonucleotides in mice. *Int. Immunopharmacol.*4, 851-862, 2004.
83. **ER.Kandimalla** & S.Agrawal. Agonists of Toll-like receptor 9. Modulation of host immune responses with synthetic oligodeoxynucleotides. In Toll-receptors (ed. Tina Rich) pp 1-32. Landes, Cambridge, UK, 2004.
82. W.Jiang, CF.ReichIII, D.Yu, **ER.Kandimalla**, S.Agrawal & DS.Pisetsky. Induction of immune activation by a novel immunomodulatory oligonucleotide without thymocyte apoptosis. *Biochem. Biophys. Res. Commun.* **318**, 60-66, 2004.
81. **ER.Kandimalla**, RK.Pandey & S.Agrawal. Hybridization-based fluorescence assay allows quantitation of single-stranded oligodeoxynucleotides in low nanomolar range. *Anal. Biochem.* **328**, 93-95, 2004.
80. DK.Agrawal, J.Edwan, **ER.Kandimalla**, D.Yu, L.Bhagat, D.Wang & S.Agrawal. Novel Immunomodulatory oligonucleotides (IMOs) prevent development of allergic airway inflammation and airway hyperresponsiveness in Asthma. *Int. Immunopharmacol.* 4, 127-138, 2004.
79. D.Wang, Y.Li, D.Yu, S.S.Song, **ER.Kandimalla** & S.Agrawal. Immunopharmacological and antitumor effects of second-generation immunomodulatory oligonucleotides containing synthetic CpR motifs. *Int. J. Oncol.* **24**, 901-908, 2004 .
78. S.Agrawal & **ER.Kandimalla**. Modulation of Toll-like receptor 9 responses through synthetic immunostimulatory motifs of DNA. *Ann. N. Y. Acad. Sci.*, **1002**, 30-42, 2003.
77. **ER.Kandimalla** & S.Agrawal. Chemistry of CpG DNA. In Curr. Prot. Nucleic Acids Chem. (Ed. S. Beucauge), pp 4.13.1-4.13.13, John-Wiley, New York, 2003.
76. **ER.Kandimalla**, L.Bhagat, FG.Zhu, D.Yu, YP.Cong, D.Wang, JX.Tang, JY.Tang, CF.Knetter, E.Lien & S.Agrawal. A dinucleotide motif in oligonucleotides shows potent immunomodulatory activity and overrides species specific recognition observed with CpG motif. *Proc. Natl. Acad. Sci. USA*. **100**, 14303-14308, 2003.
75. YP.Cong, SS.Song, L.Bhagat, RK.Pandey, D.Yu, **ER.Kandimalla** & S.Agrawal. Self-stabilized CpG DNAs optimally activate human B cells and plasmacytoid dendritic cells. *Biochem. Biophys. Res. Commun.* **310**, 1133-1139, 2003.
74. **ER.Kandimalla**, L.Bhagat, YP.Cong, RK.Pandey, D.Yu, Q.Zhao & S.Agrawal. Secondary structures in CpG oligonucleotides affect immunostimulatory activity. *Biochem. Biophys. Res. Commun.* **306**, 948-953, 2003.
73. **ER.Kandimalla**, L.Bhagat, D.Wang, D.Yu, FG.Zhu, J.Tang, H.Wang, P.Huang, R.Zhang & S.Agrawal. Divergent synthetic nucleotide motif recognition pattern: design and development of potent immunomodulatory oligodeoxyribonucleotide agents with distinct cytokine induction profiles. *Nucleic Acids Res.*, **31**, 2393-2400, 2003.
72. D.Yu, **ER.Kandimalla**, Q.Zhao, L.Bhagat, Y.Cong & S.Agrawal. Requirement of nucleobase proximal to CpG dinucleotide for immunostimulatory activity of synthetic CpG DNA. *Bioorg. Med. Chem.* **11**, 459-464, 2003.
71. **ER.Kandimalla**, FG.Zhu, L.Bhagat, D.Yu & S.Agarawal. Toll-like receptor 9: Modulation of recognition and cytokine induction by novel synthetic CpG DNAs. *Biochem. Soc. Trans.*, **31**, 654-658, 2003.
70. L.Bhagat, F.G.Zhu, D.Yu, J.Tang, H.Wang, E.R.Kandimalla, R.Zhang, S.Agrawal. CpG Penta- and Hexadeoxyribonucleotides as Potent Immunomodulatory Agents. *Biochem. Biophys. Res. Commun.* **300**, 853-861, 2003.
69. D.Yu, **ER.Kandimalla**, L.Bhagat, JY.Tang, Y.Cong, J.Tang & S.Agrawal. 'Immunomers' - Novel 3'-3'-linked CpG oligodeoxynucleotides as potent immunomodulatory agents. Nucleic Acids Res., **30**, 4460-4469, 2002.
68. D.Yu, F-G.Zhu, L.Bhagat, H.Wang, **ER.Kandimalla**, R.Zhang & S.Agrawal. Potent CpG oligonucleotides containing phosphodiester linkages: In vitro and in vivo immunostimulatory properties. *Biochem. Biophys. Res. Commun.* **297**, 83-90, 2002.
67. **ER.Kandimalla**, L.Bhagat, D.Yu, Y.Cong, J.Tang & S.Agrawal. Conjugation of ligands at the 5'-end of CpG DNA affects immunostimulatory activity. *Bioconj. Chem.* **13**, 966-974, 2002.
66. D.Yu, **ER.Kandimalla**, Y.Cong, J.Tang, JY.Tang, Q.Zhao & S.Agrawal. Design, synthesis, and immunostimulatory properties of CpG DNAs containing alkyl-linker substitutions: Role of nucleosides in the flanking sequences. *J. Med. Chem.* **45**, 4540-4548, 2002.
65. D.Yu, **ER.Kandimalla**, Q.Zhao, Y.Cong & S.Agrawal. Immunostimulatory properties of

phosphorothioate CpG DNA containing both 3'-5'- and 2'-5'-internucleotide linkages. *Nucleic Acids Res.* **30**, 1613-1619, 2002.

64. H.Wang, J.Hang, Z.Shi, M.Li, D.Yu, **ER.Kandimalla**, S.Agrawal & R.Zhang. Antisense oligonucleotide targeted to RIα subunit of cAMP-dependent protein kinase (GEM 231) enhances therapeutic effectiveness of cancer chemotherapeutic agent irinotecan in nude mice bearing human cancer xenografts: In vivo synergistic activity, pharmacokinetics and host toxicity. *Int. J. Oncol.* **21**, 73-80, 2002.
63. S.Agrawal, **ER.Kandimalla**, D.Yu, DL.Dexter, R.Ball, G.Lombardi, T.Lucas, BA.Hollister, & SF.Chen. GEM 231, A Second-Generation Antisense Agent Complementary to Protein Kinase A RIα Subunit, Potentiates Antitumor Activity of Irinotecan in Human Colon, Pancreas, Prostate and Lung Cancer Xenografts. *Int. J. Oncol.* **21**, 65-72, 2002.
62. **ER.Kandimalla**, D.Yu, & S.Agrawal. Towards Optimal Design of Second-Generation Immunomodulatory Oligonucleotides. *Curr. Opin. Mol. Ther.*, **4**, 122-129, 2002
61. S.Agrawal & **ER.Kandimalla**. Medicinal chemistry and therapeutic potential of CpG-DNA. *Trend. Mol. Med.*, **8**, 114-121, 2002.
60. BJ.Premraj, PK.Patel., **ER.Kandimalla**, S.Agrawal, RV.Hosur & N.Yathindra. NMR Structure of a 2'-5' RNA favors A-type duplex with compact C2'-endo nucleoside repeat. *Biochem. Biophys. Res. Commun.* **283**, 537-543, 2001.
59. S.Agrawal & **ER.Kandimalla**. Antisense and/or immunostimulatory oligonucleotide therapeutics. *Curr. Cancer Drug Targets*, **1**, 197-209, 2001.
58. **ER.Kandimalla** & S.Agrawal. Therapeutic Potential of Synthetic CpG DNA-Current Status and Future Directions. *I. Drugs*, **4**, 963-966, 2001.
57. D.Yu, **ER.Kandimalla**, Q.Zhao, Y.Cong & S.Agrawal. Modulation of immunostimulatory activity of CpG oligonucleotides by site-specific deletion of nucleobases. *Booorg. Med. Chem. Lett.* **11**, 2263-2267, 2001.
56. D.Yu, **ER.Kandimalla**, Q.Zhao, Y.Cong & S.Agrawal. Immunostimulatory activity of CpG oligonucleotides containing non-ionic methylphosphonate linkages. *Bioorg. Med. Chem.* **9**, 2803-2808, 2001.
55. **ER.Kandimalla**, D.Yu, Q.Zhao & S.Agrawal. Effect of chemical modifications of cytosine and guanine in a CpG-motif of oligonucleotides on immunostimulatory activity: Structure-immunostimulatory activity relationships. *Bioorg. Med. Chem.*, **9**, 807-813, 2001.
54. S.Agrawal, **ER.Kandimalla**, D.Yu, BA.Hollister, SF.Chen, DL.Dexter, TL.Alford, B.Hill, KS.Bailey, CP.Bono, DL.Knoerzer & PA.Morton. Potentiation of antitumor activity of Irinotecan by chemically modified oligonucleotides. *Int. J. Oncol.* **18**, 1061-1069, 2001.
53. SL.Shankar, S.Mani, KN.O'Guin, **ER.Kandimalla**, S.Agrawal & B.Shafit-Zagardo. Survivin inhibition induces human neural tumor cell death through caspase-independent and -dependent pathways. *J. Neurochem.* **79**, 426-436, 2001.
52. F.Ciardiello, R.Caputo, T.Troiani, **ER.Kandimalla**, S.Agrawal, J.Mendelsohn, AR.Bianco & G.Tortora. Antisense oligonucleotides targeting the epidermal growth factor receptor inhibit proliferation, induce apoptosis, and cooperate with cytotoxic drugs in human cancer cell lines. *Int. J. Cancer* **93**, 172-178, 2001.
51. Y.Lu, S.Mani, **ER.Kandimalla**, D.Yu, S.Agrawal, JC.States & DB.Bregman. The cockayne syndrome group B DNA repair protein as an anti-cancer target. *Int. J. Oncol* **19**, 1089-1097, 2001.
50. D.Yu, Q.Zhao, **ER.Kandimalla** & S.Agrawal. Accessible 5'-end of CpG-containing phosphorothioate oligodeoxynucleotides is essential for immunostimulatory activity. *Bioorg. Med. Chem. Lett.* **10**, 2585-2588, 2000.
49. S.Agrawal & **ER.Kandimalla**. Antisense therapeutics. Is it as simple as complementary base recognition? *Mol. Med. Today*, **6**, 72-81, 2000.
48. D.Yu, **ER. Kandimalla**, A.Roskey, Q.Zhao, L.Chen, J.Chen & S.Agrawal. Stereo-enriched phosphorothioate oligodeoxynucleotides: Synthesis, biophysical and biological properties. *Bioorg. Med. Chem.* **8**, 275-284, 2000.
47. **ER.Kandimalla** & S.Agrawal. 'Cyclicons' as hybridization-based fluorescent primer-probes - Synthesis, properties and application in real-time PCR. *Bioorg. Med. Chem.* **8**, 1911-1916, 2000.
46. S.Agrawal & **ER.Kandimalla**. Medicinal chemistry of antisense oligonucleotides. In Antisense Technology in the Central Nervous System, (Eds. R.Leslie, J.Hunter and H.Robertson), pp108-136, Oxford University Press, Oxford, 1999.

45. Z.Jiang, **ER.Kandimalla**, Q.Zhao, LX.Shen, A.DeLuca, N.Normano, M.Ruskowski & S.Agrawal. Pseudo-cyclic oligonucleotides: In vitro and in vivo properties. *Bioorg. Med. Chem.* 7, 2727-2735, 1999.
44. **ER.Kandimalla**, DR.Shaw & S.Agrawal. Effects of phosphorothioate oligodeoxyribonucleotide and oligoribonucleotides on human complement and coagulation. Bioorg. Med. Chem. Lett. 8, 2103-2108, 1998.
43. S.Agrawal, X.Zhang, Q.Cai, **ER.Kandimalla**, A.Manning, Z.Jiang, T.Marcel & R.Zhang. Effect of aspirin on protein binding and tissue disposition of oligonucleotide phosphorothioate in rats. *J. Drug Target.* 5, 303-312, 1998.
42. LX.Shen, **ER.Kandimalla** & S.Agrawal. Impact of mixed-backbone oligonucleotides on target binding affinity and target cleaving specificity and selectivity by E. coli RNase H. *Bioorg. Med. Chem.* 6, 1695-1705, 1998.
41. DR.Shaw, PK.Rustagi, **ER.Kandimalla**, AN.Manning, Z.Jiang & S.Agrawal. Effects of synthetic oligonucleotides on human complement and coagulation. *Biochem. Pharmacol.* 53, 1123-1132,1997.
40. **ER.Kandimalla**, G.Venkataraman, V.Sasisekharan & S.Agrawal. Single-stranded DNA and RNA targeted triplex-formation: UV, CD and molecular modeling studies of foldback triplexes containing different RNA and DNA strand combinations. *J. Biomolec. Struc. Dyn.* 14, 715-726, 1997.
39. **ER.Kandimalla**, A.Manning, Q.Zhao, DR.Shaw, RA.Byrn, V.Sasisekharan & S.Agrawal. Mixed backbone antisense oligonucleotides: Design, biochemical and biological properties of oligonucleotides containing 2'-5'-ribo and 3'-5'-deoxyribo-nucleotide segments. *Nucleic Acids Res.* 25, 370-378, 1997.
38. **ER.Kandimalla** & S.Agrawal. Mixed backbone antisense oligonucleotides containing 2'-5'-ribo and 3'-5'-deoxyribonucleosides: Synthesis, biochemical and biological properties. *Nucleic Acids Sym. Ser.* 35, 125-126, 1996.
37. **ER.Kandimalla** & S.Agrawal. Hoogsteen DNA duplexes of 3'-3' and 5'-5' attached oligonucleotides and triplex-formation with RNA and DNA pyrimidine sequences: Experimental and molecular modeling studies. *Biochemistry* 35, 15332-15339, 1996.
36. **ER.Kandimalla**, A.Manning & S.Agrawal. Single strand targeted triplex formation: Physicochemical and biochemical properties of foldback triplexes. *J. Biomol. Struct. Dyn.* 14, 79-90, 1996.
35. **ER.Kandimalla**, A.Manning & S.Agrawal. Single strand targeted triplex formation: Strand displacement of duplex DNA by foldback triplex-forming oligonucleotides. *J. Biomol. Struct. Dyn.* 13, 483-492, 1995.
34. **ER.Kandimalla**, A.Manning, G.Venkataraman, V.Sasisekharan & S.Agrawal. Single strand targeted triplex formation: Targeting purine-pyrimidine mixed sequences using abasic linkers. *Nucleic Acids Res.* 23, 4510-4517, 1995.
33. **ER.Kandimalla**, A.Manning, C.Lathan, RA.Byrn & S.Agrawal. Design, biochemical, biophysical and biological properties of cooperative antisense oligonucleotides. *Nucleic Acids Res.* 23, 3578-3584, 1995.
32. **ER.Kandimalla**, S.Agrawal, G.Venkataraman & V.Sasisekharan. Single strand targeted triplex formation: Parallel-stranded DNA hairpin duplexes for targeting homopyrimidine strands. *J. Am. Chem. Soc.* 117, 6416-6417, 1995.
31. **ER.Kandimalla** & S.Agrawal. Single strand targeted triplex formation: Destabilization of guanine quadruplex structures by foldback triplex-forming oligonucleotides. *Nucleic Acids Res.* 23, 1068-1074, 1995.
30. **ER.Kandimalla** & S.Agrawal. Destabilization of DNA guanine quadruplex structure by foldback triplex-forming oligodeoxynucleotides. *Nucleosides Nucleotides* 14, 991-995, 1995.
29. **ER.Kandimalla**, J.Temsamani & S.Agrawal. Synthesis and properties of 2'-O-methylribonucleotide methylphosphonate containing chimeric oligonucleotides. *Nucleosides Nucleotides* 14, 1031-1035, 1995.
28. **ER.Kandimalla** & S.Agrawal. Single strand targeted triplex formation: Stability, specificity and RNase H activation properties. *Gene* 149, 115-121, 1994.
27. **KE.Rao**, G.Gosselin, D.Mrani, C.Perigaud, JL.Imbach, C.Bailly, JP.Henichart, P.Colson, C.Houssier & JW.Lown. Psoralen-lexitropsin hybrids: DNA sequence selectivity of photoinduced cross-linking from MPE footprinting and exonuclease III stop assay, and mode of binding from electric linear dichroism. *Anticancer Drug Des.* 9, 221-237, 1994.

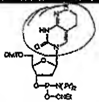
26. **KE.Rao**, S.Padmanabhan & JW.Lown. Molelcular recognition between ligands and nucleic acids: Sequence preferences and binding of pyrrolo[3,2-d] and [2,3-d]thiazole-containing lexitropsins deduced from MPE.Fe(II) footprinting. *Actual. Chim. Ther.* **20**, 159-188, 1993.
25. F.Adnet, J.Liquier, E.Taillandier, MP.Singh, **KE.Rao** & JW.Lown. FTIR study of specific binding interactions between DNA minor groove binding ligands and polynucleotides. *J. Biomol. Struc. Dyn.* **10**, 565-575, 1992.
24. D.Mrani, G.Gosselin, C.Bailly, R.Houssin, **KE.Rao**, J.Zimmermann, J.Balzarani, E.DeClercq, JP.Henichart, JW.Lown & JL.Imbach. Synthesis, DNA binding and biological evaluation of bithiazol-linked netropsin derivatives. *Eur. J. Med. Chem.* **27**, 331-344, 1992.
23. **KE.Rao** & JW.Lown. Molecular mechanism of action of saframycin antibiotics: Sequence selectivities in the covalent bonding of saframycins Mx1, Mx3, A and S deduced from MPE.Fe(II) footprinting and exonuclease stop assays. *Biochemistry* **31**, 12076-12082, 1992.
22. **KE.Rao** & JW.Lown. Lexitropsins: Sequence selective DNA binding and anticancer agents. *Trend. Org. Chem.* **3**, 141-171, 1992.
21. TA.Beerman, MM.McHugh, R.Sigmund, JW.Lown, **KE.Rao** & Y.Bathini. Effects of analogs of the DNA minor groove binder Hoechst 33258 on topoisomerase II and I mediated activities. *Biochim. Biophys. Acta* **1131**, 53-61, 1992.
20. **KE.Rao**, K.Krowicki, G.Burckhardt, C.Zimmer & JW.Lown. Molecular recognition between oligopeptides and nucleic acids: DNA binding selectivity of a series of 1,2,4-triazole-containing lexitropsins. *Chem. Res. Toxicol.* **4**, 241-252, 1991.
19. **KE.Rao**. Synthesis of distamycin and netropsin analogs. Part IV. Synthesis of bis-1,3-[3/4 (guanidinoacetamido) benzamido] benzene dihydrochlorides and bis-1,3-[4/3,5-diaminobenzamido) benzamido] benzene tetrahydrochloride. *Indian J. Chem.* **30B**, 13-17, 1991.
18. **KE.Rao**, J.Zimmermann & JW.Lown. Sequence selective DNA binding by linked bis N-methylpyrrole dipeptides: An analysis by MPE footprinting and force field calculations. *J. Org. Chem.* **56**, 786-797, 1991.
17. B.Plouvier, C.Bailly, R.Houssin, **KE.Rao**, JW.Lown, JP.Henichart & MJ.Waring. DNA sequence specific recognition by a thiazole analogue of netropsin - An MPE.Fe(II) and DNase I footprinting study. *Nucleic Acids Res.* **19**, 5821-5829, 1991.
16. TA.Beerman, JW.Woynarowski, RD.Sigmund, LS.Gawron, **KE.Rao** & JW.Lown. Netropsin and bis-netropsin analogs as inhibitors of the catalytic activity of mammalian DNA topoisomerase II and topoisomerase cleavable complexes. *Biochim. Biophys. Acta* **1090**, 52-60, 1991.
15. **KE.Rao** & JW.Lown. Molecular recognition between ligands and nucleic acids: DNA binding characteristics of analogues of Hoechst 33258 designed to exhibit altered base and sequence recognition. *Chem. Res. Toxicol.*, **4**, 661-669, 1991.
14. J.Zimmermann, **KE.Rao**, T.Joseph, AM.Sapse & JW.Lown. Amide isosteres of lexitropsins: Synthesis, DNA binding characteristics and sequence selectivity of thioformyldistamycin. *J. Biomol. Struct. Dyn.*, **9**, 599-611, 1991.
13. SM.Lal, **KE.Rao**, YN.Vaishnav, BU.Rao & V.Sasisekharan. Antivaral activity and inhibition of macromolecular synthesis of human neoplastic cells by synthetic analogues of distamycin and netropsin. *Indian J. Virol.*, **7**, 1-11, 1991.
12. WH.Gmeiner, **KE.Rao**, B.Rayner, JL.Imbach & JW.Lown. Polarity of annealing and structural analysis of the α-5'-d[TACACA]:β-5'-r[AUGUGU] hybrid resistant to RNase H mediated hydrolysis determined by high field ^{1}H, ^{13}C and ^{31}P NMR analysis. *Biochemistry* **29**, 10329-10341, 1990.
11. **KE.Rao**, Y.Bathini & JW.Lown. Synthesis of novel thiazole containing DNA minor groove binding oligopeptides related to the antibiotic distamycin. *J. Org. Chem.* **55**, 728-737, 1990.
10. Y.Bathini, **KE.Rao**, RG.Shea & JW.Lown. Molecular recognition between ligands and nucleic acids: Novel pyridine- and benzoxazole-containing agents related to Hoechst 33258 that exhibit altered DNA sequence specificity deduced from footprinting analysis and spectroscopic studies. *Chem. Res.Toxicol.* **3**, 268-280, 1990.
9. **KE.Rao** & V.Sasisekharn. Synthesis of distamycin and netropsin analogs: Part II - DNA binding bisquaternary ammonium heterocycles analogous to NSC 101327. *Indian J. Chem.* **29B**, 503-507, 1990.
8. **KE.Rao** & V.Sasisekharan. Synthesis of distamycin and netropsin analogs: Part III - Biologically active analogs of tris(m-benzamido) compound. *Indian J. Chem.* **29B**, 508-513, 1990.

7. **KE.Rao**, K.Krowicki, J.Balzarini, E.DeClercq, RA.Newman & JW.Lown. Novel linked antiviral and antitumor agents related to netropsin - 2: Synthesis and biological evaluation. *Actual. Chim. Ther.* **18**, 21-42, 1990.
6. **KE.Rao**, RG.Shea, Y.Bathini & JW.Lown. Molecular recognition between ligands and nucleic acids: DNA sequence specificity and binding properties of thiazole-lexitropsins incorporating the concepts of base site acceptance and avoidance. *Anticancer Drug Des.*, **5**, 3-20, 1990.
5. **KE.Rao** & JW.Lown. Mode of action of Saframycin antitumor antibiotics: Sequence selectivities in the covalent binding of Saframycins A and S to deoxyribonucleic acid. *Chem. Res. Toxicol.*, **3**, 262-267, 1990.
4. C.Bailly, N.Helbecque, JP.Henichart, P.Colson, C.Houssier, **KE.Rao**, RG.Shea & JW.Lown. Molecular recognition between oligopeptides and nucleic acids. DNA sequence specificity and binding properties of an acridine-linked netropsin hybrid ligand. *J. Molec. Recogn.*, **3**, 26-35, 1990.
3. **KE.Rao**, N.Ramesh, D.Choudhury, SK.Brahmachari & V.Sasisekharan. Role of the environment in the interaction of non-intercalators with Z-DNA. *J. Biomol. Struc. Dyn.* **7**, 335-345, 1989.
2. **KE.Rao**, D.Dasgupta & V.Sasisekharan. Interaction of synthetic analogues of distamycin and netropsin with nucleic acids. Does curvature of ligand play a role in distamycin-DNA interactions? *Biochemistry*, **27**, 3018-3024, 1988.
1. M.Rajagopalan, **KE.Rao**, J.Ayyer & V.Sasisekharan. Synthesis of a distamycin analogue: Tris(m-benzamido) compound. *Indian J. Chem.* **26B**, 1021-1024, 1987.

Issued/granted Patents

15. Short immunomodulatory oligonucleotides (US/7,354,907)
14. Modulation of immunostimulatory properties of oligonucleotide-based compounds by optimal presentation of 5' ends (US/7,276,489)
13. Modulation of immunostimulatory activity of immunostimulatory oligonucleotide analogs by positional chemical changes (US/7,262,286)
12. Modulation of oligonucleotide CpG-mediated immune stimulation by positional modification of nucleosides (US/7,176,296)
11. Modulation of oligonucleotide CpG-mediated immune stimulation by positional modification of nucleosides (US/7,115,579)
10. Modulation of oligonucleotide CpG-mediated immune stimulation by positional modification of nucleosides (US/7,105,495)
9. Pseudo-cyclic oligonucleobases (US/6,383,752)
8. Cooperative oligonucleotides (US/6,372,427).
7. Affinity-based purification of oligonucleotides using soluble multimeric oligonucleotides (US/5,912,332).
6. Mixed backbone antisense oligonucleotides conatining 2'-5'-ribonucleotide- and 3'-5'-deoxyribonucleotide segments (US/5,886,165).
5. Integrated oligonucleotides (US/5,739,308).
4. Triplex-forming antisense oligonucleotides having abasic linkers targeting nucleic acids comprising mixed sequences of purines and pyrimidines (US/5,693,773).
3. Pseudo-cyclic oligonucleobases (EP1086216B1)
2. Oligonucleotide alkylphosphonates and alkylphosphonothioates (EP0677056B1).
1. Foldback triplex-forming oligonucleotides (EP0680489B1)

Glen Research: P-base



Catalog Number: 10-1047-xx
Description: dP-CE Phosphoramidite

6H,8H-3,4-dihydro-pyrimido[4,5-c][1,2]oxazin-7-one,8-[(5'-dimethoxytrityl- -D-deoxyribofuranosyl),3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Formula: $C_{41}H_{50}N_5O_8P$ M.W.: 771.85 F.W.: 330.23

Diluent: Anhydrous Acetonitrile
Coupling: No changes needed from standard method recommended by synthesizer manufacturer.
Deprotection: No changes needed from standard method recommended by synthesizer manufacturer.
Storage: Refrigerated storage, maximum of 2-8°C, dry
Stability in Solution: 24 hours

Catalog Information | Material Safety Data Sheet

EXTINCTION DATA

Item	Nucleoside	Max-1 (nm)	Emax-1 (ml/µmole)	Max-2 nm	Emax-2 (ml/µmole)	E260 (ml/µmole)
10-1047	dP	294	6.7	231	7.4	2.9

LITERATURE HIGHLIGHTS

Glen Report 9.1: PROPERTIES OF OLIGONUCLEOTIDES CONTAINING THE BASES P AND K
Glen Report 8.1: NEW UNIVERSAL AND DEGENERATE BASES

DILUTION/COUPLING DATA

The table below shows pack size data and, for solutions, dilutions and approximate couplings based on normal priming procedures. Please link for more detailed usage information with the various synthesizers.

ABI 392/394									
Cat.No.	Pack Size	Grams/ Pack	0.1M Dil. (mL)	LV40	LV200	40nm	0.2µm	1µm	10µm
				Approximate Number of Additions					
10-1047-02	0.25grams	.25grams	3.24	94.67	56.8	35.5	25.82	18.93	4.73
10-1047-90	100µmoles	.077grams	1	20	12	7.5	5.45	4	1
Expedite									
Cat.No.	Pack Size	Grams/ Pack	Dilution (mL)	Molarity	50nm	0.2µm	1µm	15µm	
				Approximate Number of Additions					
10-1047-02	0.25grams	.25grams	4.83	.067	90.2	56.38	41	5.64	
10-1047-90	100µmoles	.077grams	1.5	.067	23.6	14.75	10.73	1.48	
Beckman									
Cat.No.	Pack Size	Grams/ Pack	Dilution (mL)	Molarity	30nm	200nm	1000nm		
				Approximate Number of Additions					
10-1047-02	0.25grams	.25grams	4.83	.067	91.8	57.38	41.73		
10-1047-90	100µmoles	.077grams	1.5	.067	23.2	15.75	11.45		

05/03/2010 | http://www.glenres.com/ProductFiles/10-1047.html

Exhibit 2

Agrawal dP

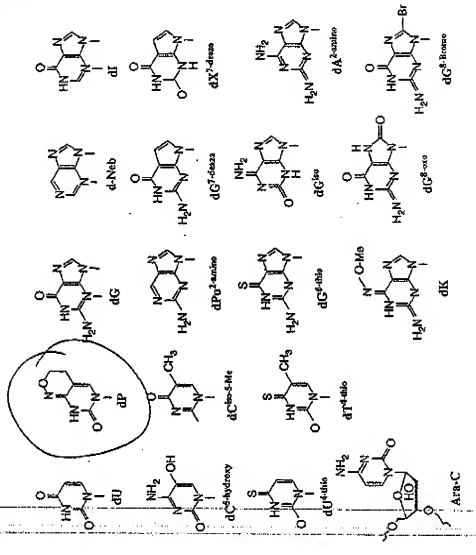


FIG. 3

Exhibit 3

Kandimalla 2001
Bioorg & Med Chem
p. 808 Fig. 2, panel 7
deoxy-P-base-nucleoside

resulted in a significant increase in immunostimulatory activity.[21] In addition, we have also shown that an accessible 5′-end, but not 3′-end, was critical for immunostimulatory activity of CpG-PS-oligos.[22]

The precise structural requirements and specific functional groups of the CpG-motif necessary for the recognition of protein/receptor factor that is responsible for immune stimulation have not yet been studied in detail. In this paper, we describe the results of a systematic study in which natural cytosine or guanine in a CpG-motif was replaced with a number of pyrimidine or purine analogues. The purpose of this study was to understand which functional groups of cytosine and guanine could be involved in the recognition of and interaction with factors responsible for immune stimulation. The in vitro and in vivo studies of CpG-PS-oligos containing modified purine bases (R) suggest that the alteration of functional groups at positions 1, 2, and 6 of guanine (see Fig. 1 for structure and numbering) significantly decreased immunostimulatory activity, while the deletion of nitrogen at the 7-position (N7) had an insignificant impact. Similarly, studies with CpG-PS-oligos containing modified pyrimidine bases (Y) suggested that the alteration of functional groups at positions 2, 3, and 4 of cytosine (see Fig. 1 for structure and numbering) significantly decreased immunostimulatory activity. Substitution of a hydrophobic methyl group at the 5-position decreased immunostimulatory activity and a hydrophilic hydroxy group at the same position did not suppress immunostimulatory activity. This is the first report of the use of chemically modified pyrimidine (Y) or purine (R) bases in place of natural cytosine or guanine, respectively, in a CpG-motif of oligos for immunomodulatory effects. In this paper, we describe the structure–immunostimulatory activity relationships of YpG- and CpR-motif-containing-PS-oligos compared with those of CpG-motif-containing-PS-oligos.

cytidine (3), deoxy-5-hydroxycytidine (4), deoxyuridine (5), deoxy-N4-ethylcytidine (6), and deoxy-8-bromoguanosine (7) (Fig. 2). The modified purine nucleobases

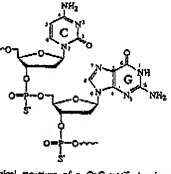


Figure 1. Chemical structure of a CpG-motif showing functional groups on cytosine and guanine that serve as hydrogen bond acceptor and donor groups.

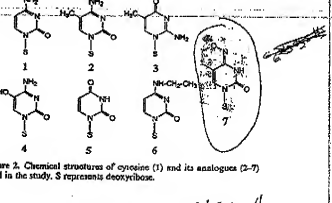
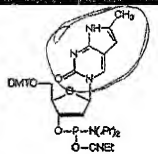


Figure 2. Chemical structures of cytosine (1) and its analogues (2–7) used in the study. S represents deoxyribose.

Exhibit 4

Glen Research: pyrrolo



Catalog Number: 10-1017-xx
Description: Pyrrolo-dC-CE Phosphorami
5'-Dimethoxytrityl-[6-methyl-pyrrolo-[2,3-d]-pyrimidine-2
2'-deoxyribonucleoside, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-p
Formula: $C_{40}H_{50}N_5O_7P$ M.W.: 767.85

Diluent: Anhydrous Acetonitrile
Coupling: Standard coupling time. Use 0.02 M Iodine for Oxidation.
Deprotection: Ammonium Hydroxide for 24 hours at room temperature.
Storage: Refrigerated storage, maximum of 2-8°C, dry
Stability in Solution: 2-3 days
Please Note: Patents Pending.

Catalog Information | Material Safety Data Sheet

EXTINCTION DATA

Item	Nucleoside	Max-1	Emax-1	Max-2	Emax-2	E260
		(nm)	(ml/µmole)	nm	(ml/µmole)	(ml/µmole)
10-1017	Pyrrolo-dC	339	2.36	229	17.5	2.41

LITERATURE HIGHLIGHTS

Glen Report 15.1: Pyrrolo-C - a novel fluorescent nucleoside
Glen Report 16.1: Minor Base and Related Novel Phosphoramidites

DILUTION/COUPLING DATA

The table below shows pack size data and, for solutions, dilutions and approximate couplings based detailed usage information with the various synthesizers.

Exhibit 5

Liu JMB 2001
p. 466
pyrrolodC

While the smaller phage polymerase holds little or no structural homology with its prokaryotic and eukaryotic counterparts, if fundamental principles of nucleic acid topology and stability govern the design of an RNA polymerase, one might expect key features of the various elongation complexes to be similar (convergently evolved). In particular, von Hippel has proposed that heteroduplex energetics is a key component of the overall stability of an elongation complex (Wilson *et al.*, 1999). Thus, a minimal heteroduplex length (and therefore, bubble size) is proposed to be essential for a stable elongation complex. Here, we probe the size of the elongation bubble in the T7 enzyme.

Although complexes containing RNA shorter than about nine or ten nucleotides cannot be stably isolated, a recent study has shown that complexes paused at positions 10 to 14 nucleotides from the start site are much more stable (Montesano *et al.*, 2000). A recent probing of similarly paused elongation complexes, using nucleases to estimate the footprint of the enzyme on the DNA and potassium permanganate to probe unpaired DNA bases within the bubble, provides evidence for a seven base-pair heteroduplex, with about a nine base open bubble (Huang & Sousa, 2000). The footprinting results in that study also illustrate clearly that probes which bind directly to the DNA to exert their effect, such as nucleases (and methy-

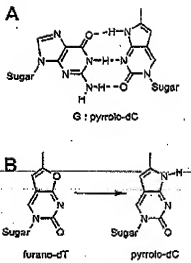


Figure 1. (a) During deprotection of a synthetic oligonucleotide, incorporated furano-dT (left) is quantitatively converted to pyrrolo-dC (right). (b) Structure of pyrrolo-dC (right) base-paired with guanine.

Exhibit 6